How to Make a Relationship Last: Release Sites with Different Levels of Commitment

Stimulus-induced changes in synaptic strength endure for periods ranging from minutes to days. In this issue of *Neuron*, Kim et al. show that two distinct types of synaptic change—synaptic vesicle movement into previously empty varicosities and formation of new varicosities—may determine intermediate- versus long-term synaptic facilitation.

One of the most striking aspects of a neuron that differentiates it from most other cell types is the sheer number and variety of its subcellular compartments. For example, neuronal somata, axons, dendrites, nodes of Ranvier, and growth cones express very different patterns of proteins that are required for their specific functions. To a first approximation, a neuron can be considered to comprise a number of very different cell types linked to a common nucleus. With an appropriate pattern of stimulation, a neuron may selectively modify the morphological and electrical properties of one or more of these specific compartments, resulting in changes in animal behavior and learning that may endure for periods ranging from only a few minutes up to the life of the animal.

One clear and extensively investigated example of changes in neuronal properties that underlie a change in behavior is the connection between the sensory and motor neurons that underlie the gill-withdrawal reflex in *Aplysia*. In response to repeated stimulation or, in vitro, to repeated applications of the neurotransmitter 5-hydroxytryptamine (5-HT), this synaptic connection undergoes facilitation, and the time course of this facilitation depends on the intensity and duration of the stimulus (Ghirardi et al., 1995; Sutton et al., 2001). Kim et al. (2003 [this issue of *Neuron*]) have now found that the duration of synaptic facilitation may be determined by differential regulation of two different neuronal subcompartments, mature functional synaptic terminals and "empty" axonal varicosities.

Kim et al. (2003) used three different imaging procedures, coupled with electrophysiological recordings, to track changes in the presynaptic terminals of the sensory neurons following facilitation of this synaptic connection in culture. A fluorescent dye was used to visualize the entire outline of a sensory neuron and of its axonal arbors that contact the motor neuron. The location of synaptic vesicles was imaged by transfecting the sensory neurons with a synaptic vesicle protein, synaptophysin tagged with green fluorescent protein. Finally, synaptic endings were determined as functional or nonfunctional by transfecting with synapto-pHluorin, which localizes to the lumenal face of synaptic vesicles, where its fluorescence is suppressed by the acidic environment of the vesicle. When a vesicle undergoes exocytosis, the protein is exposed to the more basic external medium, and the fluorescence of this protein increases (Miesenbock et al., 1998).

Release of neurotransmitter typically occurs at axonal swellings, termed varicosities, in contact with the processes of a postsynaptic neuron. An examination of the varicosities of the *Aplysia* sensory neurons in contact with the motor neuron revealed that a significant proportion of them are "empty," i.e., they do not contain synaptic vesicles (Figure 1). Rapid filling of many of these empty varicosities can, however, be achieved by stimuli that produce facilitation of transmission that lasts for at least 30 min. One of the stimuli used by Kim et al. (2003) comprised four consecutive applications of a low concentration of 5-HT. This neurotransmitter is known to activate both the protein kinase C and the cyclic AMP-dependent protein kinase signaling pathways. The four applications of 5-HT produce only an intermediate-term synaptic facilitation that endures for about 3 hr. The filling of the empty varicosities with synaptic vesicles, measured 3 hr after stimulation, is unaffected by an inhibitor of protein synthesis, implying that simply a rearrangement of preexisting components has occurred. As the facilitation waned with time, the newly filled varicosities again lost their vesicular content and, after 24 hr, these varicosities had returned to their empty state.

It takes more than an accumulation of synaptic vesicles to form an active release site. It also requires the machinery for their release, including voltage-dependent calcium channels and the presynaptic plasma membrane proteins that control exocytosis (Ahmari et al., 2000; Garner et al., 2002). Kim et al. (2003) suggest that many of these may also be recruited en masse to the previously empty varicosities. The rapid directed movement of vesicles and other components required for release to construct a functioning release site may be a relatively general phenomenon in neurons both in the developing brain and in the adult. For example, related morphological changes occur in peptidergic neurons of *Aplysia* upon activation of protein kinase C and the cyclic AMP-dependent proteins kinase, the two pathways that are activated by 5-HT in the sensory neurons (Knox et al., 1992). In the peptidergic neurons, this leads both to a change in the rate of movement of the secretory organelles and to their movement into previously organelle-free domains, including the actin-rich lamellipodia of growth cones at the distal end of neurites. As a result, these are transformed into bulb-like endings engorged with vesicles. Simultaneously, the activation of protein kinase C results in the appearance of new voltage-dependent α1A calcium channel subunits at these distal endings, probably by their insertion from a pool of subplasmalemmal vesicles (Knox et al., 1992; White et al., 1998).

More intense stimulation of the sensory neurons, achieved in vitro using five consecutive pulses of a much higher concentration of 5-HT, produces long-term facilitation that lasts at least 24 hr. At first, the changes that occur in the terminals of the sensory neurons resemble those produced by the weaker stimulus that produces only intermediate-term facilitation. In particular, rapid filling of previously empty varicosities with synaptic vesicles can be detected as soon as 30 min after stimulation. Nevertheless, there appears to be an important difference in the mechanism by which these previously empty vesicles become filled. In contrast to the weaker stimulus, which after 3 hr produced filling that required only

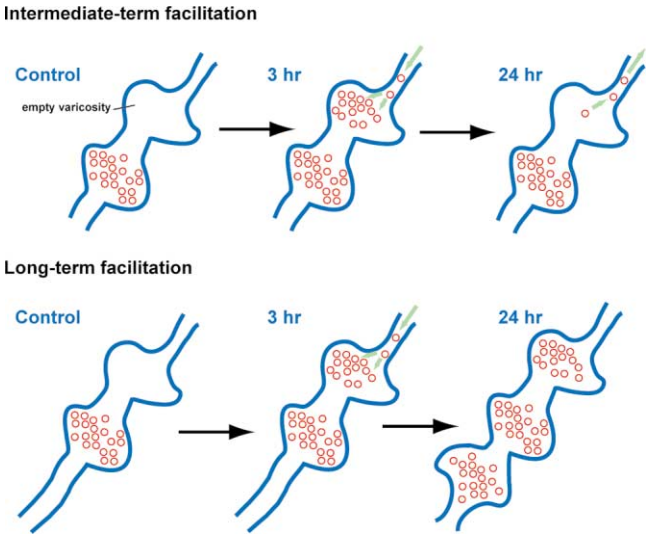

Figure 1. A Model for Changes in Sensory Neuron Axonal Varicosities during Intermediate-Term and Long-Term Facilitation of Synaptic Transmission

preexisting components, the filling of the empty varicosities that is measured 3 hr after the stronger stimulus is completely blocked by preventing new protein synthesis. It is not yet known how the mechanism that causes a rearrangement of preexisting components to fill empty varicosities is suppressed by the stronger stimulus to become supplanted by a protein synthesis-dependent mechanism for filling. Nevertheless, with the stronger stimulus, there is no loss of vesicles from the release sites over time, and the varicosities remain filled even after 24 hr.

The long-term filling of preexisting varicosities cannot account fully for long-term facilitation. The stronger pattern of stimulation that induces long-term facilitation also produces a second form of morphological rearrangement that is the major source of new release sites detected at 24 hr. Beginning about 12 hr after stimulation, entirely new varicosities come into being. These bear a full complement of vesicles and are capable of releasing them. Many of these new varicosities appear to be formed by budding off from preexisting filled varicosities. These findings on *Aplysia* sensory neurons indicate that neurotransmitter release may occur from two distinct synaptic compartments, a labile compartment that can be rapidly and reversibly remodeled into an active release site and a stable compartment that, under appropriate stimulation, may undergo fission to produce new stable synaptic contacts.

The coexistence of two distinct compartments with different long-term stability may be a common feature of other synapses, perhaps even those that are not traditionally considered to be modified rapidly by experience. As one example, the end-bulbs and calyces of Held are giant nerve terminals in the auditory brainstem of mammals. Their role is to provide very secure and precisely timed synaptic excitation to their postsynaptic targets, and they have been widely used to investigate mechanisms of synaptic transmission (Schneggenburger et al., 2002). Yet at the ultrastructural level, even these terminals may have both labile and more structurally stable elements. The individual synaptic elements at the central regions of these terminals are characterized by a distinct cytoskeletal specialization. An array of filaments link mitochondria to the presynaptic plasma membrane, and chains of vesicles are aligned on these filaments. (Tolbert and Morest, 1982; Rowland et al., 2000). In contrast, close to the peripheral regions of such terminals, growth cones can be detected even in adult animals (Jones et al., 1992). Although synaptic vesicles and active zones can be found at the peripheral sites, these lack the characteristic array of filaments with their associated mitochondria (Rowland et al., 2000). It is possible, therefore, that the cytoskeletal organization of the central regions represents stable release sites, while the synaptic contacts closer to the periphery of the terminal represent more labile sites that can be recruited or suppressed according to the ongoing pattern of synaptic traffic.

The existence of distinct types of synaptic endings, which elect to function either as "full-time" or "part-time" release sites, raises novel questions. How and when do the "empty" varicosities form? What are the signals that eventually grant "tenure" to a newly formed functional release site? Work with nonneuronal cells has provided insights into the mechanisms that regulate the stability, survival, and death of an entire cell. It remains to be determined whether any of these mechanisms have been coopted into the regulation of autonomous neuronal subcompartments such as synaptic endings.

Leonard K. Kaczmarek
Departments of Pharmacology and
Cellular and Molecular Physiology
Yale University School of Medicine
333 Cedar Street
New Haven, Connecticut 06520

Selected Reading

Ahmari, S.E., Buchanan, J., and Smith, S.J. (2000). Nat. Neurosci. *3*, 445–451.

Garner, C.G., Zhai, R.G., Gundelfinger, E.D., and Ziv, N.E. (2002). Trends Neurosci. *25*, 243–250.

Ghirardi, M., Montarolo, P.G., and Kandel, E.R. (1995). Neuron *14*, 413–420.

Jones, D.R., Hutson, K.A., and Morest, D.K. (1992). Synapse *10*, 291–309.

Kim, J.-H., Udo, H., Li, H.-L., Youn, T.Y., Chen, M., Kandel, E.R., and Bailey, C.H. (2003). Neuron *40*, this issue, 151–165.

Knox, R.J., Quattrocki, E.A., Connor, J.A., and Kaczmarek, L.K. (1992). Neuron *8*, 883–889.

Miesenbock, G., De Angelis, D.A., and Rothman, J.E. (1998). Nature *394*, 192–195.

Rowland, K.C., Irby, N.K., and Spirou, G.A. (2000). J. Neurosci. *20*, 9135–9144.

Schneggenburger, R., Sakaba, T., and Neher, E. (2002). Trends Neurosci. *25*, 206–212.

Sutton, M.A., Masters, S.E., Bagnall, M.W., and Carew, T.J. (2001). Neuron *31*, 143–154.

Tolbert, L.P., and Morest, D.K. (1982). Neuroscience *7*, 3053–3067.

White, B.H., Nick, T.A., Carew, T.J., and Kaczmarek, L.K. (1998). J. Neurophysiol. *80*, 2514–2520.